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APPLICATION FOR UNITED STATES LETTERS PATENT

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TITLE: SUSTAINED RELEASE FORMULATIONS

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Sustained Release Formulations

Background of the Invention

Field of the Invention

This invention relates to sustained release preparation of growth hormone, especially human growth hormone.

Description of Related Disclosures

Sustained release delivery systems are of interest because they can improve patient compliance, tolerability, product performance, and expand the market potential for a product. The SABER system is a novel injectable liquid non-polymeric drug delivery system (Smith and Tipton (1996) Pharmaceutical Research 13(3):300). The SABER system, which stands for Sucrose Acetate isoButyrate Extended Release, is composed of sucrose acetate isobutyrate (SAIB) and a plasticizing solvent. SABER is injected as a low viscosity liquid that increases rapidly in viscosity after injection. The resulting high viscosity matrix is adhesive, biodegradable and biocompatible.

Clinically, rhGH is administered daily in growth hormone deficient (GHD) patients. To decrease the dosing frequency and increase patient compliance several sustained release formulations are under development. Recently the FDA approved the first rhGH sustained release formulation. This formulation allows patients to decrease their dosing interval from daily to once or twice per month depending on rhGH requirements. However, the approved Depot formulation releases 10-20% of the encapsulated protein in the first two days leading to high rhGH serum levels (Johnson et al., (1996) Nature Medicine (2):795-799).

Summary of the Invention

The present invention provides novel non-polymeric sustained release formulations of growth hormone.

Brief Description of the Drawings

Figure 1: The structure of sucrose acetate isobutyrate is shown in Figure 1.

Figure 2: The effect of solvent ratio (Figure 2A) and loading (Figure 2B and 2C) on the release of hGH from sucrose acetate isobutyrate formulations containing ethanol.

Figure 3: The effect of solvent on the release of rhGH from sucrose acetate isobutyrate formulations.

Figure 4: The effect protein formulation has on the relapse of rhGH from sucrose acetate isobutyrate formulations.

Figure 5: Protein integrity after relapse from sucrose acetate isobutyrate formulations determined by native size exclusion chromatography and reverse phase HPLC.

Figure 6: Effect of solvent quality on stability of rhGH released from sucrose acetate isobutyrate formulations containing reagent and USP grade benzyl benzoate.

Figure 7: The effect a chelating agent (EDTA) has on the relapse of zinc complexed rhGH from sucrose acetate isobutyrate formulations.

Figure 8: The effect buffer exposed surface area and sucrose acetate isobutyrate buffer ratio have on the relapse of rhGH from sucrose acetate isobutyrate formulations.

Figure 9: rhGH serum levels after subcutaneous administration of rhGH sucrose acetate formulation (SD rats 6/group, 15 mg/Kg).

Detailed Description of the Preferred Embodiments

Modes for Carrying out the Invention

The purpose of this study was to evaluate the release of recombinant human growth hormone (rhGH) from a non-polymeric sucrose acetate isobutyrate sustained release system.

The system comprised sucrose acetate isobutyrate (SAIB) and a solvent. Two spray freeze dried formulations of rhGH were evaluated, rhGH in sodium bicarbonate and rhGH complexed with zinc. The rhGH powders were homogenized with various systems at two different protein loads(5 and 15% w/v). The release rate and protein stability was monitored by reverse phase-HPLC, size exclusion chromatography and BCA for 28 days. The effect of zinc and surface area on release rate and protein stability was also investigated.

The in vitro results for the zinc complexed rhGH indicated a very low burst from 0.1 (SAIB:Ethanol) to 2.2 % (SAIB:Miglyol) followed by protein release over 28 days. The release rates and total protein released by the different preparations varied widely. The high protein load (15%) and the low protein load (5%) released approximately the same amount of protein indicating that the surface area of the sucrose acetate

isobutyrate:solvent/protein mix proved to be an important factor in the initial burst and the release rate. In vitro experiments that increased the surface area of the sucrose acetate isobutyrate:solvent/protein in contact with the release medium resulted in increased bursts of 1 to 4% with a higher total percentage of released protein. The bicarbonate rhGH suspension had a higher initial burst (7 to 14%) and released more protein in 28 days when compared to the zinc complexed rhGH suspension.

Changing the solvent polarity, the ratio of solvent to SAIB, and the addition of zinc can modify the release rate of the rhGH from sucrose acetate isobutyrate:solvent systems. These results demonstrate that the sucrose acetate isobutyrate:solvent delivery system is capable of providing sustained release of intact rhGH in vitro.

Sucrose acetate isobutyrate extended release systems are described in U.S. patent no. 5,747,058, for example, the disclosure of which is specifically incorporated herein by reference.

The growth hormone (GH) is preferably human growth hormone (hGH), preferably biologically active non-aggregated hGH. According to the present invention the GH is complexed with at least one type of multivalent metal cation, preferably having a valence of +2 or more, preferably from a metal cation component of the formulation.

Suitable multivalent metal cations include biocompatible and non-toxic metal cations. A preferred metal cation component for GH is Zn +2. Typically, the molar ratio of metal cation component to GH is between 1:1 and 100:1, preferably, between 1:1 and 20:1 and preferably between 1:1 and 10:1.

The following examples are offered by way of illustration and not by way of limitation. The disclosures of all citations in the specification are expressly incorporated herein by reference.

EXAMPLES

EXAMPLE I

METHODS

Preparation of zinc complexed rhGH: A 20 mg/ml rhGH solution in 25 mM sodium bicarbonate was complexed with zinc at a rhGH:zinc ratio of 10:1. The rhGH/zinc suspension was spray

freeze dried to create a fine powder that is approximately 70% rhGH by weight.

Preparation of bicarbonate rhGH: A solution of approximately 5 mg/ml rhGH in 10 mM ammonium bicarbonate was lyophilized to produce an excipient free powder.

SAIB/rhGH suspension preparation: The rhGH SABER suspensions were prepared by mixing rhGH powders with SABER formulations using a shear homogenizer. Release Rate Determination: 0.2 mL of each rhGH/SAIB suspension was added to eppendorf tubes in duplicate, then 0.5 mL of release medium (50 mM HEPES, 10 mM KCl, 0.1% NaN3, pH 7.2) was added above the suspension. The eppendorf tubes were incubated at 37 deg. C and sampled at various time points. At each time point, 0.5 mL of release medium was removed and 0.5 mL of fresh release medium added. Collected samples were stored at -70 deg. C prior to analysis. The release samples were analyzed for protein concentration and protein quality.

BCA Assay: The BCA assay in a microtiter plate format was used to determine the protein concentration of the release samples. rhGH protein standards were prepared in release medium at 0,0.005,0.01,0.02,0.05,0.2,0.5 g/ml. 0.02 mL of each blank, standards, and release samples were mixed with 0.2 mL of the BCA working reagent in a microtiter plate. The microtiter plate was incubated at 37 deg. C for 1 hr and the absorbance determined at 562 nm using a microtiter plate reader. The protein concentrations of the release samples were determined from the standard curve using a four parameter non-linear curve fit. The amount of oxidized variants in the rhGH release samples was determined by RP-HPLC. This assay was run using a 4.6 X 15 cm, 8 mm, 300 angstrom PLRPS column held at room temperature. The mobile phase A contained 50mM NaH2PO4, pH 7.0 and mobile phase B contained 20% propanol in acetonitrile. The separation was isocratic at 49% (B) and the eluent was monitored for absorbance at 214 nm.

Size Exclusion Chromatography was used to determine amount of monomer present in the release samples. This assay was run using a 7.8 X 300 mm TSK 2000SWXL column held at room temperature. The mobile phase used was 50mM NaH2PO4, 150 mM NaCl pH 7.2 with a flow rate of 1.0 ml/min and a run time of 20 min. 10 g protein was injected and the eluent monitored for absorbance at 214 nm.

In vivo pharmacokinetics of rhGH were determined in after SC injection of rhGH SABER formulations (SAIB:Benzyl alcohol; 85:15 w/w and SAIB:Benzyl benzoate; 70:30 w/w) in Sprague Dawley (SD) rats. Serum rhGH levels were determined by ELISA (Genentech) with an assay detection limit of 0.1 ng/mL.

RESULTS AND DISCUSSION

Solvent ratio

The effect of the SAIB/solvent ratio on protein released was examined by plotting the cumulative release for rhGH in SAIB:ethanol ratios, 85:15, 75:25, and 50:50 (w/w). This plot is shown in Figure 2A. The 85:15, 75:25, and 50:50 w/w ratio resulted in a 10%, 13%, and 26% release of the protein at 28 days. The SAIB/solvent ratio is a factor in release rate, but it does not effect the initial burst for the SAIB:ethanol formulations.

SABER solvent type

The effect of solvent on the rate of release from SABER is shown in Figure 3. All SAIB/solvent preparations show a low initial burst of rhGH in the first day and protein release out to 28 days. The rhGH/SAIB:miglyol suspension was the only sample with a poor release curve. The total amount of protein released over the 28 days for all samples was no higher than 13% of the total protein load. This result was expected due to the lack of enzymatic degradation in these in vitro experiments.

Loading

The release results for all SAIB/solvent preparations and both protein loads are detailed in Figure 2B-C. Ideally a one month sustained release system should have an initial burst of approximately <10% and an average daily release of 3%. The results for the SABER with rhGH show a burst from 0.1 to 2.2%, with an average daily release over 28 days from 0.1 to 0.9%. These values are extremely low but expected due to the lack of in vitro degradation of SABER.

Formulation

The effect of zinc on rhGH release from SABER was evaluated by comparing release rates of zinc complexed rhGH and lyophilized rhGH in bicarbonate from SABER. 5% w/v suspensions were prepared using two SAIB/solvent preparations, benzyl benzoate, and ethanol. The release curves are shown in Figure 4. The bicarbonate rhGH produces a higher initial burst than the zinc complexed rhGH for both SABER preparations. The initial burst for the bicarbonate rhGH from SAIB:ethanol is 6.53% compared to 0.53 % for the zinc complexed rhGH. The initial burst from SAIB:benzyl benzoate is 14.64 % for the bicarbonate rhGH compared to 1.06% for the zinc complexed rhGH. The daily release and the overall total protein released is also much higher for the bicarbonate rhGH. These results indicate that excipients such as zinc can affect protein release from SABER. This effect may be due to differences in particle morphology or more likely differences in protein solubility. Zinc complexed rhGH has lower solubility than the bicarbonate formulation. The integrity of the released protein was determined by RP-HPLC and SEC. The results indicate a decrease in native protein over time (Figure 5). This decrease was most pronounced in protein released from SABER formulations containing benzyl benzoate and ethanol. Protein released from the 5% load formulations was less native than protein released from the 15% load formulations.

This may be due to a decrease in the protein:solvent ratio in the 5% load formulations, leading to higher solvent exposure in the release medium. During the course of these experiments several grades of benzyl benzoate were used (reagent grade and USP grade). Samples from experiments using these solvent grades were tested for oxidation (RP-HPLC) and aggregation (SEC). The results show protein released from the SABER formulations containing USP grade benzyl benzoate were less degraded than protein released from reagent grade benzyl benzoate (figure 6). After 21 days the amount of rhGH monomer remaining was over 90% for the USP grade benzyl benzoate formulation compared to 75% for the reagent grade formulation. The reversed phase results also show an improvement in protein quality with the USP grade benzyl benzoate. At 21 days 80% of the main peak remained compared to 60% seen with the reagent grade solvent. The purity of solvent used in SABER formulations has a direct effect on protein quality and thus should be monitored.

To determine the effect zinc had on the protein release rate, zinc complexed GH and bicarbonate rhGH were mixed with two SABER formulations containing ethanol and benzyl benzoate as solvents. In vitro release experiments were carried out using an EDTA containing release medium (50 mM HEPES, 10 mM KCl, 50 mM EDTA, 0.1% NaN3, pH 7.2). These results are summarized in Figure 7. The presence of EDTA in the release medium increased both the initial burst and the overall release for both rhGH SABER formulations.

Surface area

Exposed solvent accessible surface area and SABER:buffer ratio appeared to influence release of rhGH from SABER formulations (Figure 8). When a larger surface area and lower SABER:buffer ratio (> buffer volume) was used more rhGH was released. This result indicates that both exposed surface area

and SABER:buffer ratio should be controlled during in vitro experiments.

Pharmacokinetics

In vivo pharmacokinetics show SABER formulations are able to deliver rhGH for prolonged periods of time with a fairly low initial burst (Figure 9). However, SABER solvent properties play a large role in the release mechanism. The SABER formulation containing benzyl benzoate released >80 % of available loaded material in the first 48 hrs while the benzyl alcohol formulation delivered target (10 ng/mL) levels of rhGH for the duration of these studies. When compared to control microspheres the benzyl alcohol formulation had a significantly lower initial burst yet maintained similar serum levels for 7 days.

CONCLUSIONS

In vitro release kinetics are dependent on SAIB/Solvent type, SAIB/Solvent ratio, excipients, release medium, and surface area.

The quality of the released protein is dependent upon the type of solvent and purity of solvent used in the SABER preparation. The rhGH SABER formulations can provide a low burst, sustained release system for delivery of rhGH. However in vivo kinetics could depend on protein formulation and SABER solvent choice.